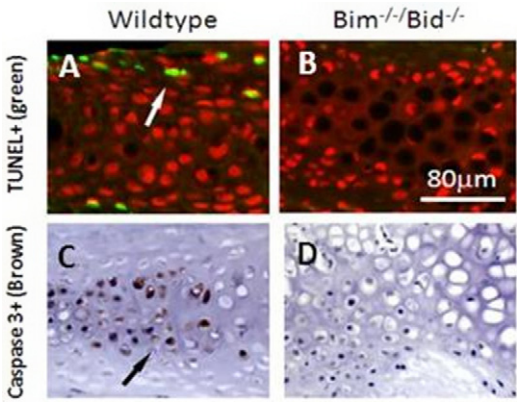

Fig. 2.

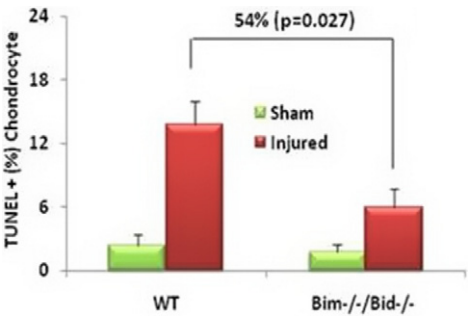

Fig. 3.

Conclusion: Our findings indicate that a deletion of Bim/Bid can reduce but not prevent chondrocyte apoptosis in response to mechanical injury. Our results are consistent with previous finding where a nitric oxide scavenger was able to reduce the incidence of chondrocyte apoptosis after traumatic injury. Since Bim (mitochondrial intrinsic) pathway has been shown to play a role in mediating nitric oxide induced apoptosis it is assumed that the Bim deletion also works through this mechanism. Additional studies are ongoing to determine whether these findings are due to a delay in the occurrence of apoptosis or long-term (>48 hours) prevention of chondrocyte apoptosis.

62
PERIOSTIN, AN OSTEOBLAST STIMULATING FACTOR, REGULATES CARTILAGE METABOLISM VIA MMP-13 ACTIVATION

M. Attur[1], G. Palmer[1], Y. Tachida[2], S. Kumakura[2], K. Shimada[2], S.B. Abramson[1]. [1]*NYUHosp. for Joint Diseases, New York, NY, USA;* [2]*Daiichi-Sankyo Co. Ltd., Tokyo, Japan*

Purpose: Periostin (POSTN), a gamma-carboxylated extracellular matrix protein originally identified as an osteoblast stimulating factor. This study investigates its expression in OA cartilage and regulation of chondrocyte metabolism.
Methods: Cartilage slices were obtained from the advanced OA patients (age 45–80 years) undergoing knee replacement surgery. Non-arthritic knee cartilages were obtained from autopsy patients within 24h (NDRI, Philadelphia). Predesigned TaqMan PCR primers were purchased from Applied Biosystems. Lentiviral shRNA targeting POSTN were purchased from Sigma. Matrix metalloproteinases proMMP-1 and proMMP-13 ELISA kits were from R&D Systems. Degradation products of type II collagen was measured using CTX II-based assay kit.
Results: We studied the expression of POSTN in pools (n = 10) of OA and age-matched non-diseased cartilage using U133 Affymetrix microarray. POSTN was overexpressed 2–4 fold in OA cartilage ($p < 0.035$) compared to non-diseased controls. Verification by real-time PCR confirmed POSTN upregulation. Surgical-induction of OA in rats by anterior cruciate ligament transection (ACLT) and destabilization of the medial meniscus (DMM) models also significantly increased POSTN (3–11 fold) from 2–8 weeks after surgery. In OA chondrocytes isolated from human tibial cartilage, POSTN mRNA levels, determined by qPCR, were inhibited 3-fold in the presence of inflammatory cytokines IL-1β and TNFα. In contrast, TGFβ-1 (2 ng/ml) significantly increased POSTN expression (2–40-fold). In functional assays, exogenously added (1–30 ug/ml) or overexpression of POSTN significantly increased MMP-13 expression and activity ($p < 0.02$) in primary human OA, rat and bovine chondrocytes. Conversely; knock down of endogenous POSTN using targeted lentiviral shRNAs significantly decreased MMP-13 expression in the presence of TGF-b1. In OA cartilage explants cultures, PSTN increased cartilage degeneration, evidenced by increased release of collagen (C1, 2C) and GAG fragments in culture supernatants. To determine whether PSTN is a hypertrophic marker of chondrocytes we examined its expression in chondrogenesis assays using human bone marrow-derived MSCs and immature murine costal chondrocytes undergoing maturation. In both assays PSTN expression was upregulated in a time-dependent manner, and expression coincided with MMP-13, Alkaline Phosphatase and F-spondin. These findings suggest that PSTN expression is associated with chondrocyte terminal differentiation.
Conclusions: Together, these studies indicate that PSTN is a marker of OA cartilage and chondrocyte hypertrophy. In OA, PSTN may contribute to disease pathogenesis by promoting cartilage degradation via induction of MMP-13.

63
NATURAL HISTORY OF BONE AND CARTILAGE CHANGES IN OSTEOARTHRITIS IN GENETIC STRAINS OF MICE

S. Hashimoto, M.F. Rai, J.M. Cheverud, L.J. Sandell. *Washington Univ. Sch. of Med., St. Louis, MO, USA*

Purpose: Emerging evidence indicates that 50–75% of variation in OA is genetic, however, little evidence is available on the genes that cause OA or protect from OA. It has been demonstrated that specific strains of mice (MRL/MpJ and DBA/1) are protected from post-traumatic arthritis in young animals and age-related OA in older animals.
In the present study, selected recombinant inbred (RI) lines of SM/J mice and LG/J were used; strains 6 and 33. RI strains are genetically identical but each strain is a different recombination of the parental genotypes, so we can predict that their outcomes are due to different composition of the genome. Strain 6 and 33 were chosen because they represent extremes of healing ability in the RI population. Preliminary data indicated that strain 6 could heal an ear hole punch while strain 33 could not. The aim of this study is to compare the strain-dependent development of OA following DMM surgery, assessed by measurements of cartilage and bone over time. We also correlated the development of OA with ear tissue regeneration potential and ability to repair cartilage.
Methods: Selected RI strains (strain 6 and 33) were subjected to destabilization of medial meniscus (DMM) at 10 weeks of age in right knee joint by transection of the medial meniscotibial ligament. Cartilage degeneration was evaluated with histological sections stained with Toluidine blue and scored with scoring system at 2, 4, and 8 weeks after surgery (n = 8 at each time point). Bone changes were analyzed with micro-computed tomography (micro-CT) scanner at the same time-points as histological evaluation. The following morphometric parameters of the tibial subchondral plate were calculated for subchondral bone thickness, and trabecular bone compartments: trabecular bone volume fraction, trabecular thickness and trabecular connective density.

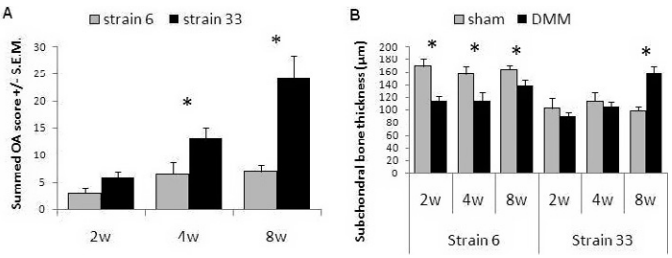

Fig. 1. (A) histological OA score of strains 6 and 33. (B) Subchondral bone thickness in medial tibial plateau of strains 6 and 33. (*$p < 0.05$).

Results: Histological sections showed more cartilage degeneration in strain 33 than strain 6 mice after DMM surgery. For the summed OA score, strain 33 mice developed OA in a time-dependent manner and significantly higher grade of OA than strain 6 mice after 4 and 8 weeks of DMM surgery (Figure 1A). Strain 33 showed subchondral bone thinning in early time points (2 and 4 weeks), however the subchondral bone

significantly thickened compared to sham knee joints at 8 weeks after surgery. In contrast, strain 6 mice showed thinning at all time points (Figure 1B). Strain 6 mice showed significantly lower bone volume fraction at all time points, while in strain 33 mice bone volume was lower only at 4 weeks and reversed from 4 to 8 weeks. Trabecular thickness was less in strain 6 mice at all time points, but only at 4 weeks in strain 33 mice with thickening at 8 weeks. The trabecular connective density, which calculates the number of trabecular bone connections, showed strain 33 mice looses trabecular bone at 4 weeks and thickens by 8 weeks after surgery, while trabecular bone loss was observed in all time points in strain 6 mice.

Conclusions: Strain 33 mice, which have poor healing ability for soft tissues like ear hole, demonstrated a natural history of OA development: they initially have bone loss, then cartilage loss followed by bone sclerosis in the late stage of OA. In contrast, strain 6 mice, which have a good healing ability in tissue, were protected against OA and showed bone loss in all time points. These results suggest that cartilage and bone changes associated with OA development are linked each other and suggest that cartilage loss leads to subchondral bone thickening. In other studies currently underway in our laboratory, we have found that strain 6 can repair a full thickness cartilage injury, while strain 33 cannot repair its cartilage (Table 1). Therefore, our studies demonstrate a significant correlation between ear punch healing, cartilage repair and resistance to OA in these genetic strains of mice.

Property	Strain 6	Strain 33
Osteoarthritis	Protected	Developed
Bone loss in early stage	Yes	Yes
Bone thickening in late stage	No	Yes
Healing ear punch	Yes	No
Cartilage regeneration	Yes	No

64

ORAL CALCITONIN DEMONSTRATED SYMPTOM-MODIFYING EFFICACY AND INCREASED CARTILAGE VOLUME: RESULTS FROM A 2-YEAR PHASE 3 TRIAL IN PATIENTS WITH OSTEOARTHRITIS OF THE KNEE

M.A. Karsdal[1], P. Alexandersen[2], M.R. John[3], J. Loeffler[3], M. Arnold[3], M. Azria[3], I. Byrjalsen[1], B.J. Riis[1], C. Christiansen[1], CCBR 2301 Investigators. *[1]Nordic BioSci., Herlev, Denmark; [2]Orla Lehmannsgade, Vejle, Denmark; [3]Novartis Pharma, Basel, Switzerland*

Purpose: To evaluate the symptom- and structure-modifying efficacy and safety of oral salmon calcitonin (oCT) formulated with a 5-CNAC carrier (a molecule based on Eligen® technology from Emisphere), in patients with moderate to severe knee pain and structural damages classified as Kellgren-Lawrence 2–3 due to osteoarthritis (OA).

Methods: A total of 1169 men and women aged 50–80 years with a mean BMI of 28.9 kg/m^2 who had painful OA of the knee with structural manifestations were enrolled in this multi-center, double-blind, randomized placebo-controlled study. Patients received (1:1) oCT 0.8 mg twice daily or placebo for 24 months; rescue medication was allowed in both arms. The primary efficacy endpoints were joint-space width (JSW), WOMAC pain and function visual analogue scale scores after 24 months in the signal knee. Secondary endpoints included but were not limited to WOMAC stiffness, patient and physician global assessments, and knee cartilage degradation. Safety endpoints were adverse events (AEs) and tolerability.

Results: Baseline values were well matched between the two groups. In ITT analysis at 24 months, oCT treatment did not have an effect on JSW (p = 0.97; progression was −0.2 mm in both groups). However, oCT increased cartilage volume by a mean 4.8% (0.73%) [*vs* 2.5% (0.61%) in placebo group, p = 0.012]. Furthermore, oCT resulted in significant changes (*vs* placebo) in WOMAC pain [−115.7 (4.9) *vs* −94.9 (4.8) mm; p = 0.002], function [−338.7 (16.7) *vs* −283.0 (15.8) mm; p = 0.013] (see Figure) and stiffness [−44.1 (2.2) *vs* −32.6 (2.1) mm; p < 0.001] scores (numbers in parentheses are standard errors). Improvement was also obtained in the oCT group (*vs* placebo) in VAS 24 hour pain (p = 0.018), patient global assessment (p = 0.008) and physician global assessment (p = 0.014). The discontinuation rate was 33% for oCT *vs* 23% for placebo treatment. Drug-related AE discontinuations occurred in 19.5% oCT group (*vs* 5.8% in placebo group), mostly happening early in the oCT group then parallel to placebo. The most common AEs in the oCT group (*vs* placebo) were hot flush (17.8 *vs* 4.1%), nausea (14 *vs* 3.1%), dyspepsia (10.1 *vs* 4.5%) and diarrhoea (9.6 *vs* 4.3%), and accounted for most discontinuations. Immunogenicity was noted in 17.4% (oCT) *vs* 1.6% (placebo) at 24 months.

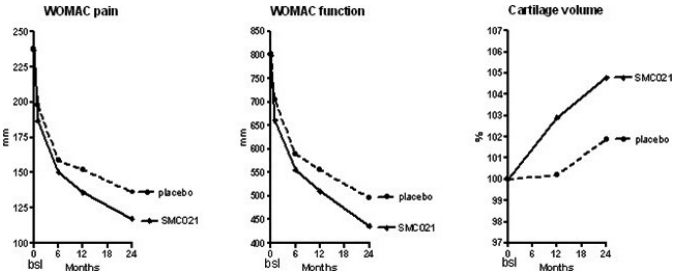

Figure: Select efficacy variables: mean changes over two years.

Conclusions: Twice daily oCT over 2 years resulted in a significant symptom-modifying efficacy in patients with painful knee OA as assessed by WOMAC pain, physical function, and stiffness scores. Although improvement on the primary endpoint of JSW was not reached, there was an increase in cartilage volume *vs* placebo indicating some structure-modifying efficacy. Safety and tolerability of oCT was largely in line with previous clinical experience with calcitonins.

65

A RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, MULTICENTER STUDY OF RHFGF18 ADMINISTERED INTRAARTICULARLY USING SINGLE OR MULTIPLE ASCENDING DOSES IN PATIENTS WITH PRIMARY KNEE OSTEOARTHRITIS (OA), NOT EXPECTED TO REQUIRE KNEE SURGERY WITHIN 1 YEAR

R. McPherson[1], K. Flechsenhar[2], S. Hellot[2], F. Eckstein[3,4]. *[1]PAREXEL Early Phase, Port Elizabeth, SOUTH AFRICA; [2]Merck Serono S.A. (an affiliate of Merck KGaA, Darmstadt, Germany), Geneva, Switzerland; [3]Paracelsus Med. Univ., Salzburg, Austria; [4]Chondrometrics GmbH, Ainring, Germany*

Purpose: There are no disease-modifying OA drugs (DMOAD) – a continuing unmet need. In OA, levels of growth factors (key regulators of chondrocyte activity) decline with age, thus reducing cartilage maintenance and ability to resist mechanical stress. Fibroblast growth factor 18 (FGF18) is a chondrogenic factor that promotes chondrocyte proliferation and stabilization of the 'healthy' anabolic chondrocyte phenotype. This Proof of Concept (PoC) study in OA patients evaluated the effects of intraarticular (i.a.) rhFGF18 injection after single ascending dose (SAD) and multiple ascending dose (MAD) regimens.

Methods: Eligible patients were ≥40 y with symptomatic primary femorotibial OA of the target knee and radiological disease (Kellgren-Lawrence grades 2 or 3), with no major knee surgery planned for ≥1 y after first injection of study drug; stable oral OA treatments were permitted. Patients received rhFGF18 or placebo, randomized 3:1 per cohort. Study drug was injected in 10 μg, 30 μg, or 100 μg doses either once (SAD) or once weekly for 3 weeks in 2 treatment cycles (MAD). Follow-up and safety reviews occurred weekly over the 4 weeks postinjection (both cohorts) and at weeks 8, 13, 14, 15, 17, 26, 39 and 52 postinjection (MAD cohorts). Efficacy (MAD cohorts) included assessment of knee cartilage in selected regions of interest by MRI exam, joint space width (JSW) of target knee on X-ray, and function and pain in target knee (WOMAC scores). MRI was performed with coronal SPGR sequences at baseline, 12, 26, and 52 weeks; X-ray was performed in the semiflexed position at baseline and 52 weeks. All cohorts were assessed for local and systemic safety. SAD cohorts were assessed for systemic exposure to rhFGF18.

Results: In SAD cohorts, 18 patients received rhFGF18; 6 received placebo. In MAD cohorts, 126 received rhFGF18; 42 received placebo. Age range was 41.4–84.9 y; the BMI range was 15.6–49.0 kg/m ^{2}. As measured in MAD cohorts, there was a significant dose-dependent anabolic effect of rhFGF18 on cartilage: for the total femorotibial cartilage volume at 12 months, compared with placebo (−112.69±470.16 mm^3) cartilage loss slowed with the lowest dose (−45.20±1117.3 mm^3), stopped with the intermediate dose (−1.09±189.93 mm^3) and reversed with the highest dose (52.68±175.7 mm^3; overall p-value=0.0251). Effects